25 p

SEMI-ANNUAL PROGRESS REPORT
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MONOCLONAL ANTIBODIES DIRECTED AGAINST SURFACE MOLECULES OF MULTICELL SPHEROIDS

Andrew O. Martinez, Ph.D.
Department of Life Sciences
University of Texas at San Antonio
San Antonio, Texas 78249

Introduction

The objective of this project is to generate a library of monoclonal antibodies (MAbs) directed against surface molecules of tumor and transformed cells grown as multicell spheroids (MCS). These MCS are highly organized, 3-dimensional multicellular structures which exhibit many characteristics of *in vivo* organized tissues which are not found in conventional monolayer or suspension culture. In brief, MCS combine the relevance of organized tissues with *in vitro* methodology making the MCS a good model system to study the interactions of mammalian cells, and thereby provide a functional assay for surface adhesion molecules.

This project also involves investigations of cell-cell interactions in a gravity-based environment. It will provide an important base of scientific information for future comparative studies on the effects of hypergravity and simulated microgravity environments on cell-cell interactions. This project also has the potential to yield important materials (e.g., cellular products) which may be useful for the diagnosis and/or treatment of certain human diseases. Moreover, this project supports the training of one undergraduate and one graduate students; thus, it will also assist in developing a pool of future scientists with research experience in gravitational biology research.

Research Progress Summary

The aims for this reporting period were: (1) to continue expansion, cloning, and characterization of MAbs; (2) to continue functional assays with MAbs; and (3) to initiate immunoprecipitation and Western blot analysis of surface molecules using MAbs from our library.

Two new MAbs directed against surface molecules of human neuroblastoma cells were isolated and cloned. The MAbs (GMJ-1, and GMJ-2) are of the IgG1 Kappa isotype. Various other hybridomas from the same fusion with different binding patterns are currently being cloned and characterized. MAbs directed against surface molecules of human glioblastoma cells were also generated and isolated. Binding patterns of selected MAbs are being characterized using immunofluorescence microscopy.

The panel of MAbs directed against surface molecules of WI38SV40 cells were further characterized on two chinese hamster ovary (CHO) cell lines since in the last progress report we noted that two of the MAbs (WSJ-3,4) exhibited slightly higher binding on CHO cells. This observation is of interest since these MAbs do not bind to any other rodent cell lines tested thus far. In addition, recent results showed that five MAbs directed against WI38SV40 surface antigens (MTS1.2, WSJ-4,5,7,9) also exhibited slightly higher binding on the CHO Pro-5 cell line, and only one MAb (WSJ-5) exhibited slightly higher binding on CHO Spinner (aggregation-deficient) cells. Three MAbs (WSJ-5,7,9) exhibited slightly higher binding to B14I50 (hamster) mutant ConA R-2DF-F1 cell line.

A study comparing MAb binding on normal WI38 fibroblasts of different passage (age) levels was initiated the reporting period. Preliminary results showed that early-passage cells exibited a lower binding intensity than did either mid-passage or late passage WI38 cells. Further comparative studies utlizing a wider range of passage levels will be conducted during the next six-month period.

The BSG-1 MAb reported previously was tested in functional assays on the 2DF*F1 cells (compaction-deficient mutant of B14I50 cells). Preliminary results showed that after 24 hr of incubation of 2DF*F1 cells in the presence of the MAb, an increase in cell aggregation, as

compared to the control, was observed.

Preliminary Western blot analysis of surface molecules of WI38SV40 cells was also initiated during the reporting period. Commercial surface proteins and antibodies were used to determine optimal experimental conditions. In addition, a radiolabeled centrifugal assay to measures cell-cell adhesion is currently being developed. This assay will be utilized in functional studies utilizing MAbs to quantitate their effects on cell-cell interactions of WI38SV40 and other cells.

A rotary cell culture sytem was recently purchased to study the effects of MAbs on cell-cell interaction in a simulated microgravity environment. Currently, optimal conditions and reagent concentrations of reagents are being determined.

In addition, I spent 10 weeks during the summer conducting research in the Life Sciences Division at the NASA-AMES Research Center in Moffet Field, California. This research was conducted in the laboratory of Dr. Rosalyn Grymes and focused on the effects of mechanical forces on the orientation response of cultured mammalian cells. A summary of this work is included in the Appendix.

Future Plans

The specific aims for the next six-month period are: (1) to continue the cloning, expansion, and characterization of MAbs; (2) to continue immunoprecipitation and Western blot analysis of surface molecules with selected MAbs; (3) to continue experiment preparation for utilizing the rotary cell culture system for testing for testing the effects of MAbs on cell-cell interations in a simulated microgravity environment; and (4) to continue optimization of the radiolabeled centrifugal assay to quantitate cell-cell adhesion.

In addition, I plan to return to NASA-AMES in summer of 1995 to continue my studies on the effects of mechanical force on the behavior of mammalian cells. In particular, I would like to study the effect of mechanical force on cytoskeletal elements such as actin filaments, microtubules and intermediate filaments, and the role of integrins and cadherins in mammalian cell alignment in response to applied mechanical force.

APPENDIX

APPENDIX

FLOW CYTOMETRY SUMMARY

ANTIBODY	CELL LINE	AVG. MEAN*	AVG. NET FLUORESCENCE*
MTS 1.2	WI38sv40	505.8	308.6
	IMR90sv40	587.6	425.2
	IMR90 P7	361.6	136.2
	IMR90 P10	388.0	160.8
BSG-1	WI38sv40	493.5	296.2
	IMR90sv40	563.9	401.5
	IMR90 P7	360.2	134.7
	IMR90 P10	382.9	155.7
WSJ-2	WI38sv40	489.2	292.0
	IMR90sv40	561.3	381.4
	IMR90 P7	347.4	121.8
	IMR90 P10	364.9	137.8
WSJ-3	WI38sv40	479.6	282.2
	IMR90sv40	550.9	388.6
	IMR90 P7	331.4	105.9
	IMR90 P10	367.6	140.4
WSJ-4	WI38sv40	474.9	277.6
	IMR90sv40	570.0	407.6
	IMR90 P7	340.0	114.5
	IMR90 P10	365.4	138.2
WSJ-5 (CS)	WI38sv40	569.7	372.4
	IMR90sv40	661.9	499.5
	IMR90 P7	535.5	310.0
	IMR90 P10	615.0	387.8
WSJ-6 (CS)	WI38sv40	527.6	330.2
	IMR90sv40	634.6	472.2
	IMR90 P7	568.7	343.2
	IMR90 P10	641.2	414.0

JORDANAGE1 FLOW CYTOMETRY SUMMARY CONT.

ANTIBODY	CELL LINE	AVG.MEAN*	AVG. NET FLUORESCENCE*
WSJ-7	WI38sv40	469.2	271.9
	IMR90sv40	563.0	400.6
	IMR90 P7	338.2	118.0
	IMR90 P10	356.0	128.8
WSJ-8	WI38sv40	497.4	300.0
	IMR90sv40	550.0	387.6
	IMR90 P7	339.0	113.6
	IMR90 P10	360.0	132.8
WSJ-9	WI38sv40	485.5	288.2
	IMR90sv40	540.3	377.9
	IMR90 P7	330.0	104.5
	IMR90 P10	340.8	113.7

*** All numbers are linear**

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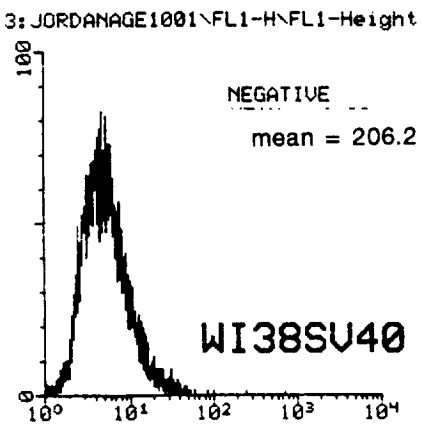

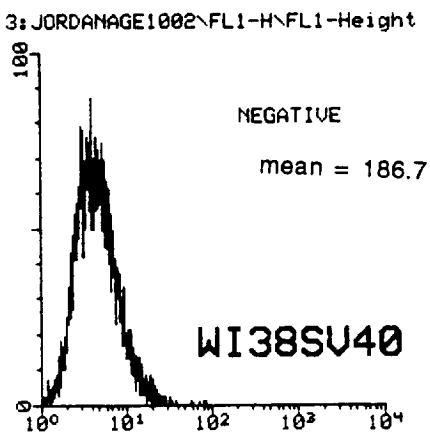

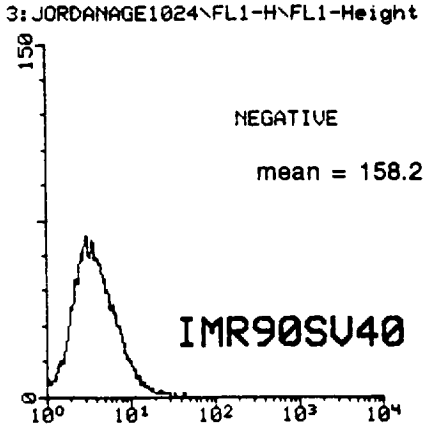

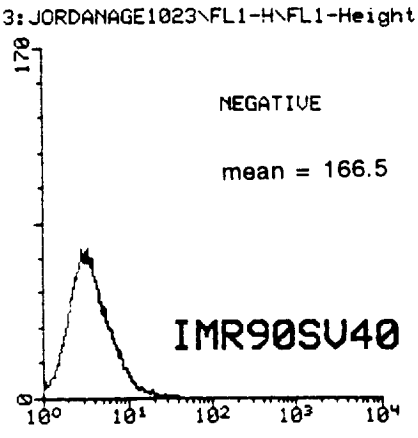

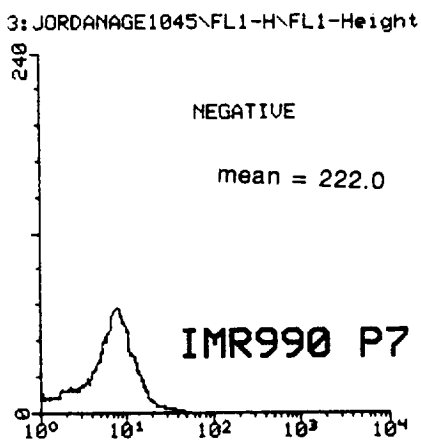

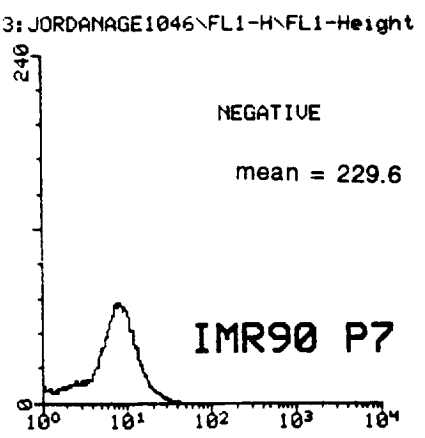

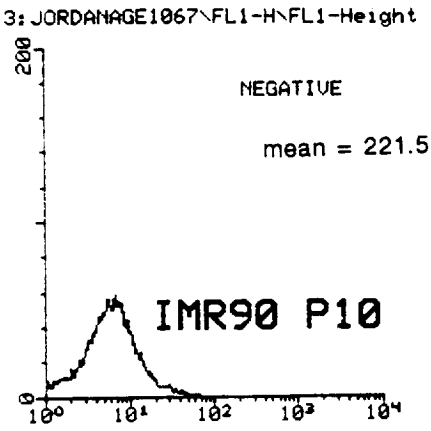

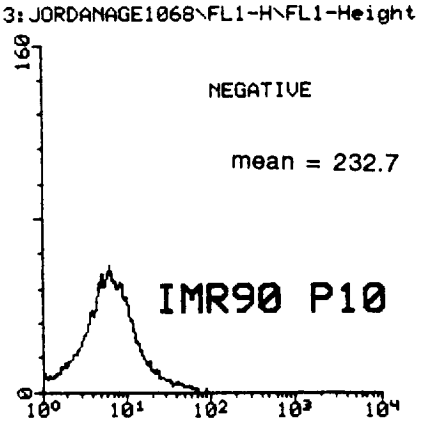

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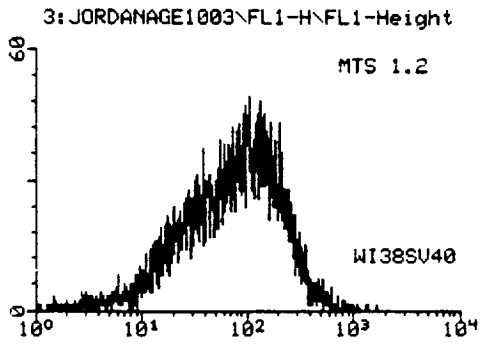

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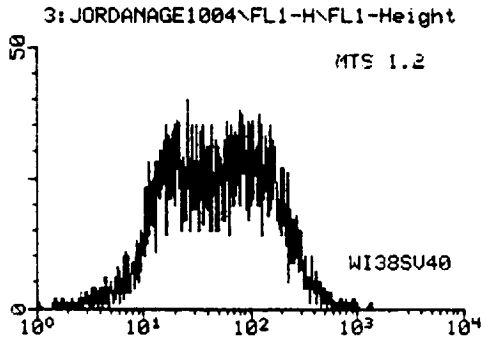

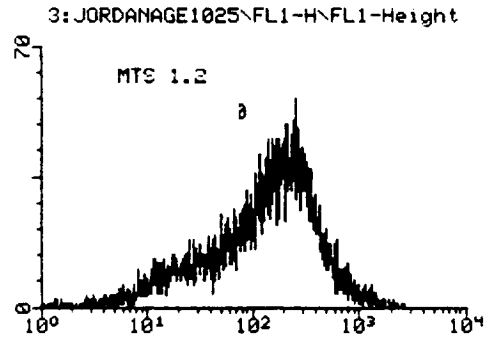

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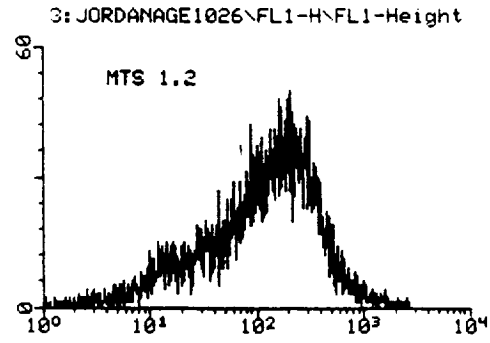

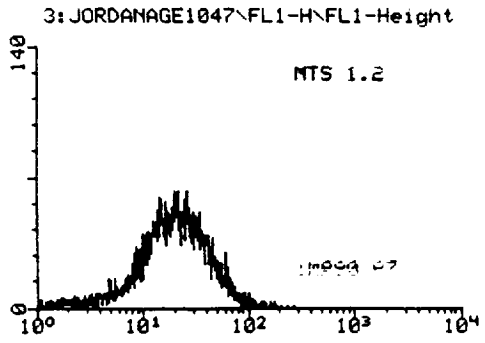

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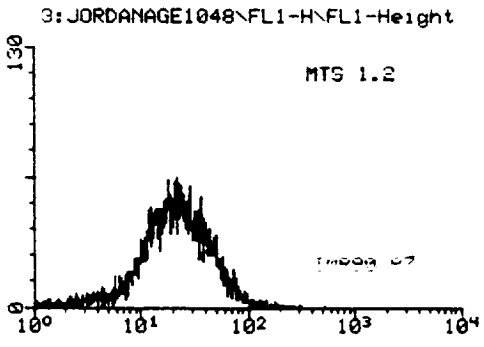

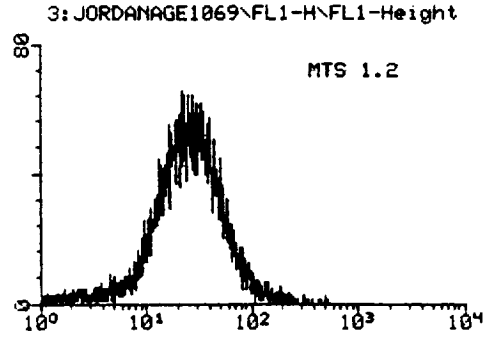

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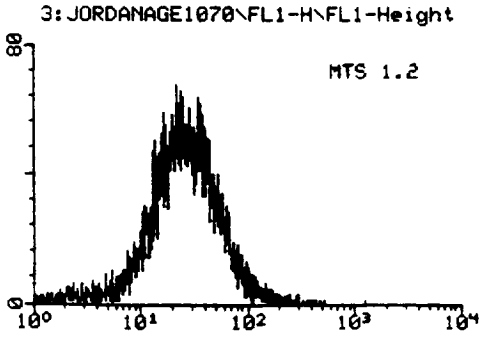

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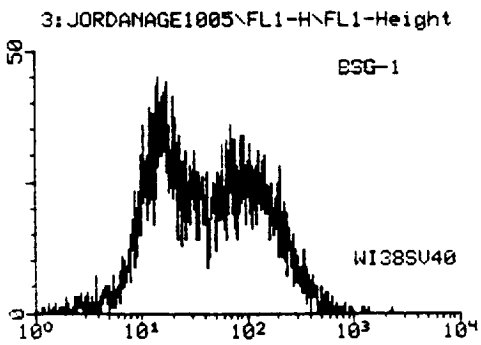

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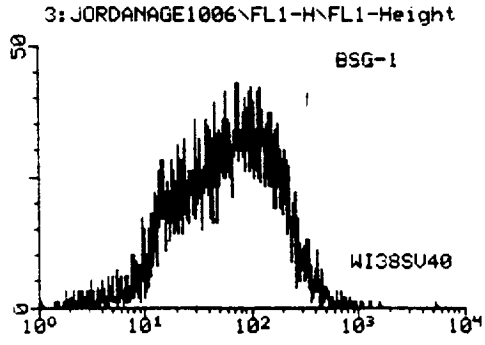

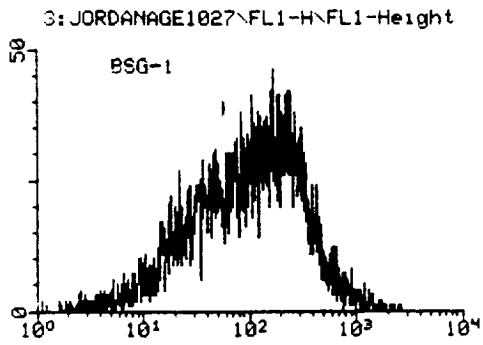

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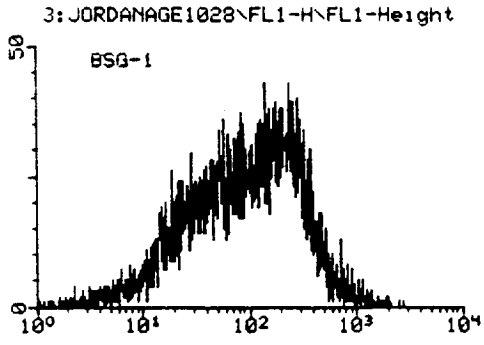

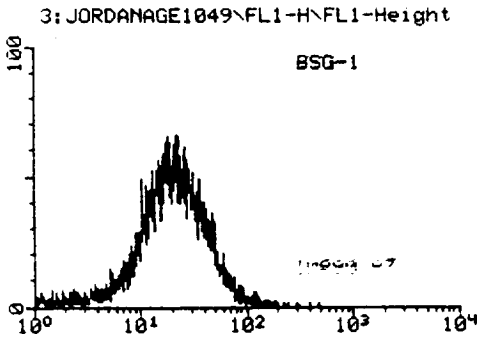

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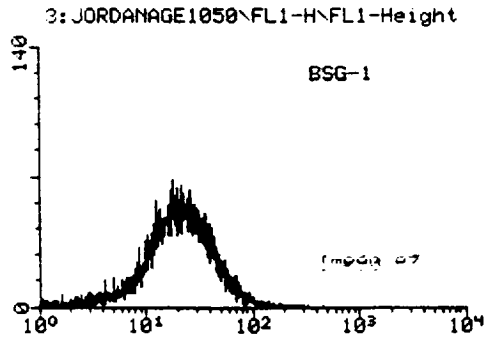

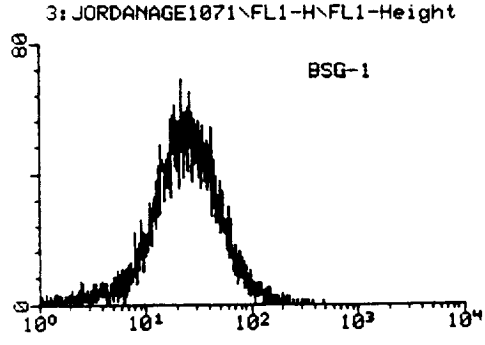

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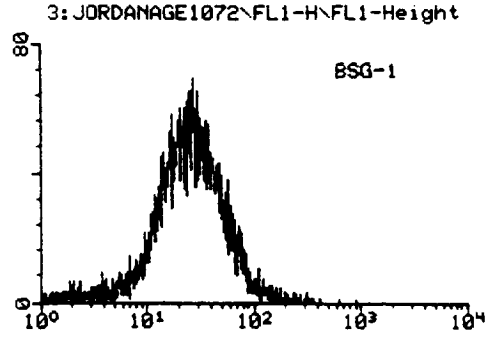

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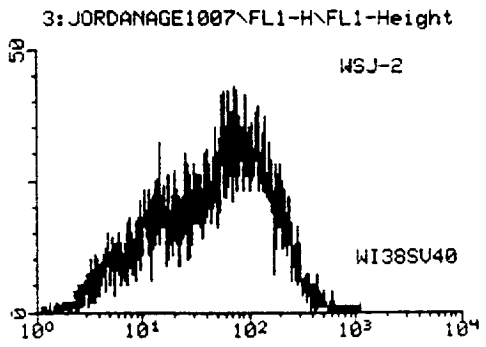

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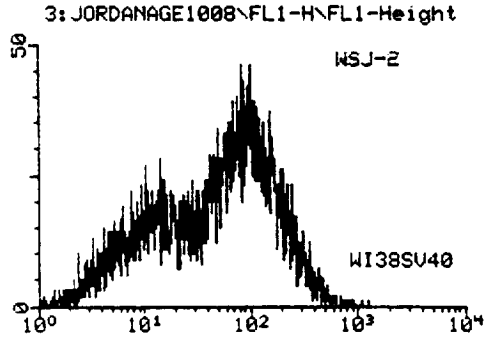

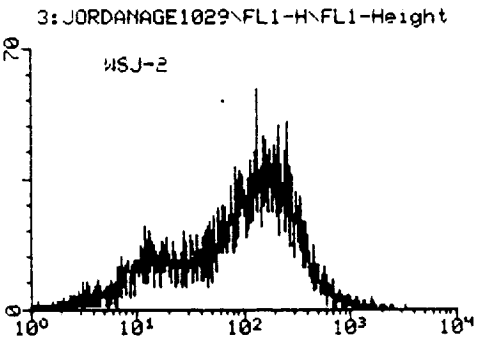

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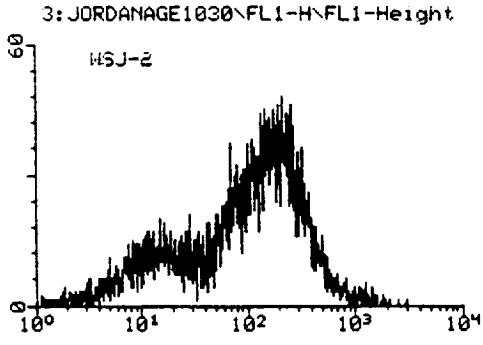

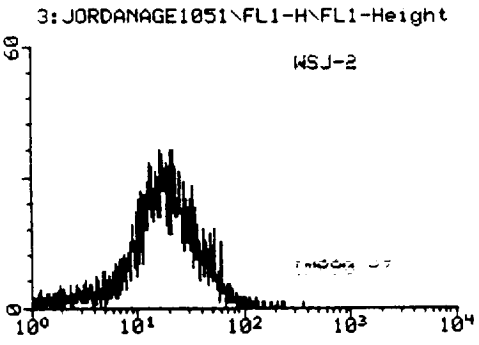

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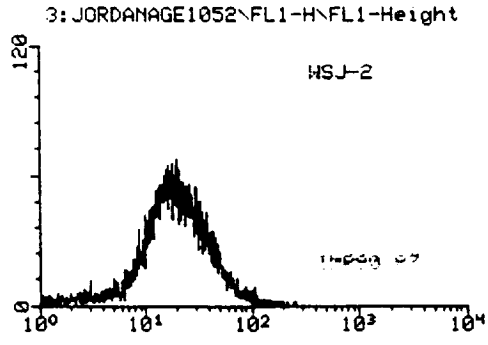

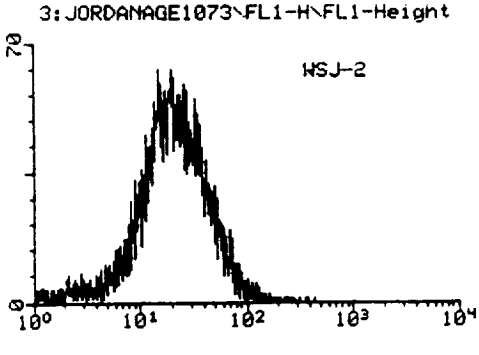

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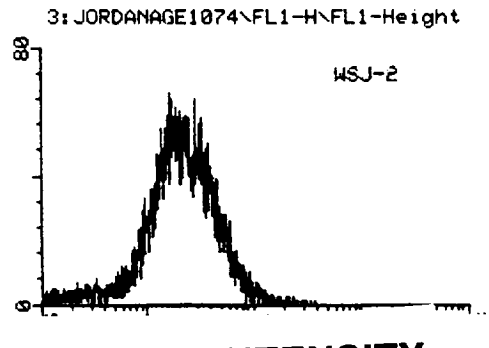

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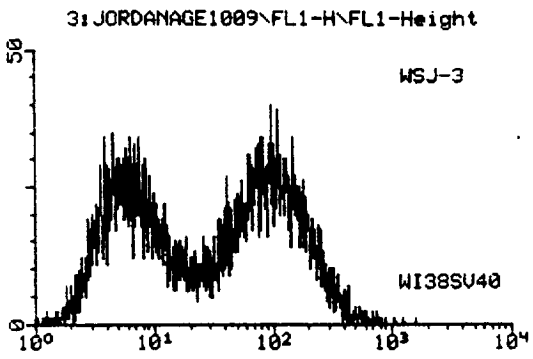

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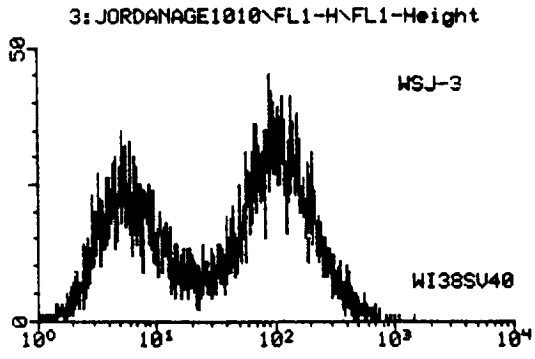

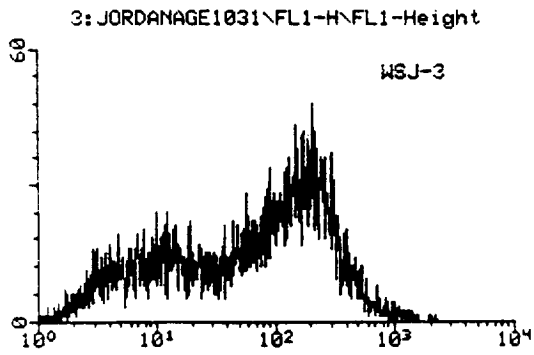

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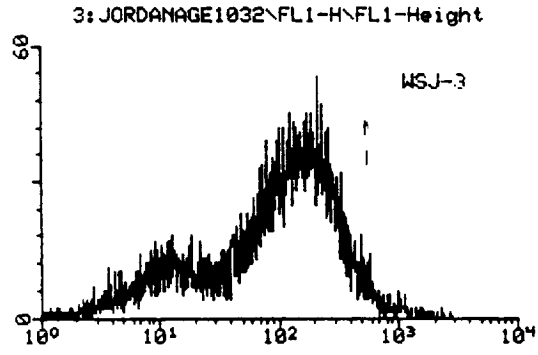

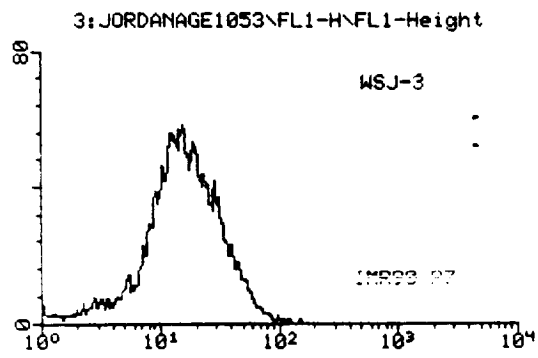

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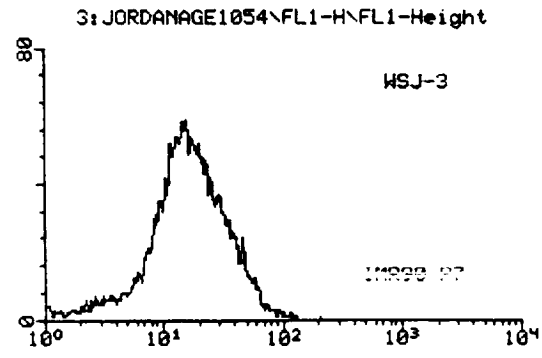

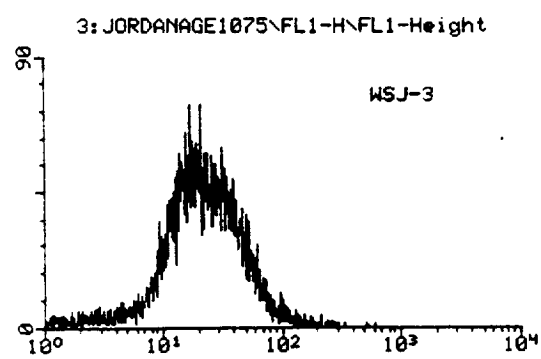

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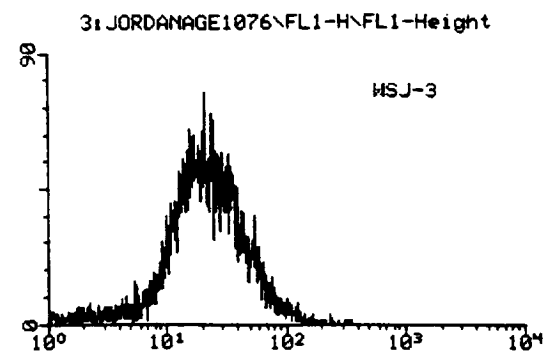

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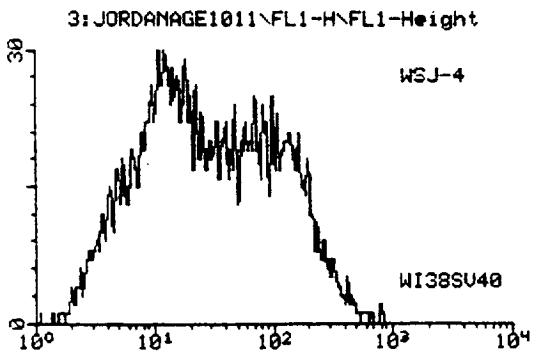

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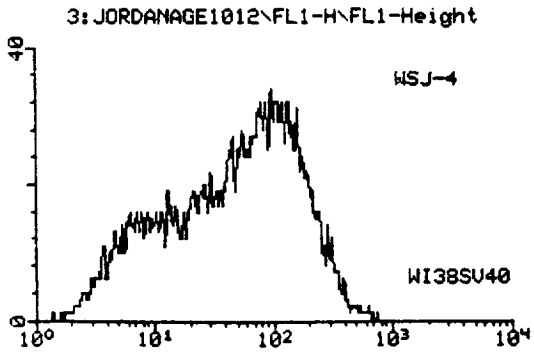

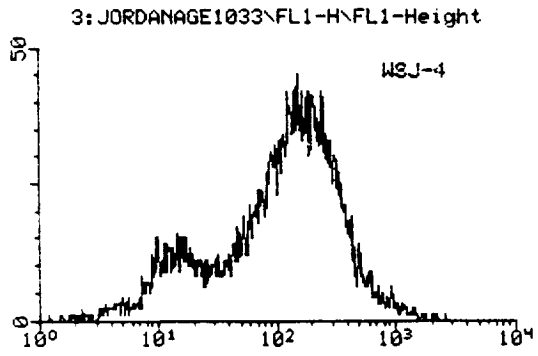

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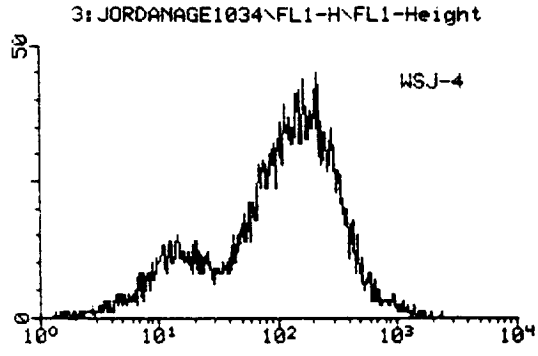
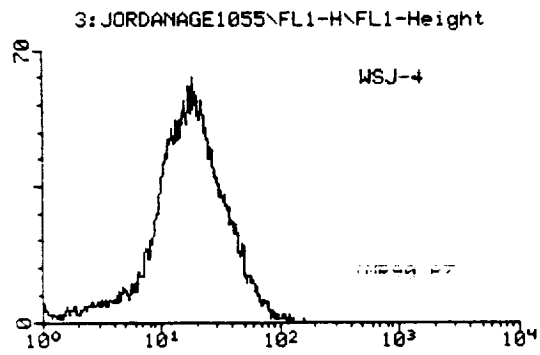
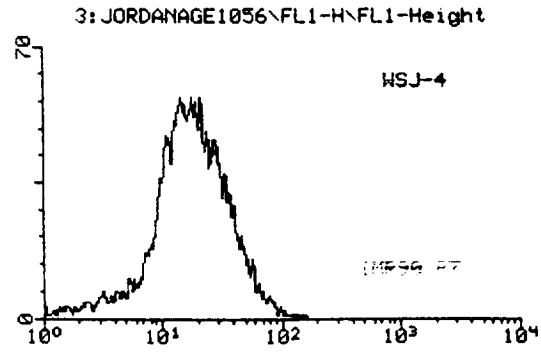
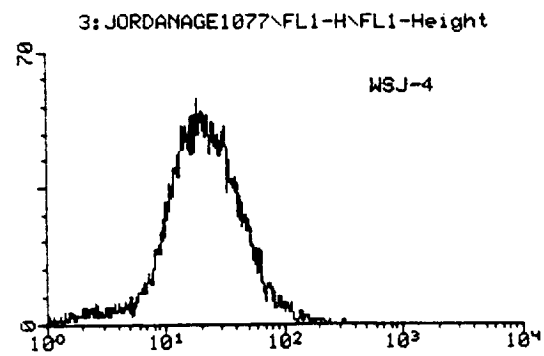


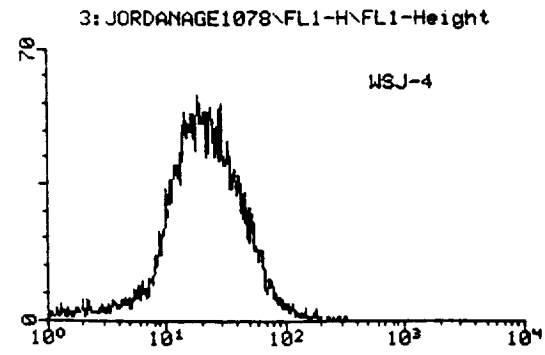


mean = 340.0
NF = 114.5





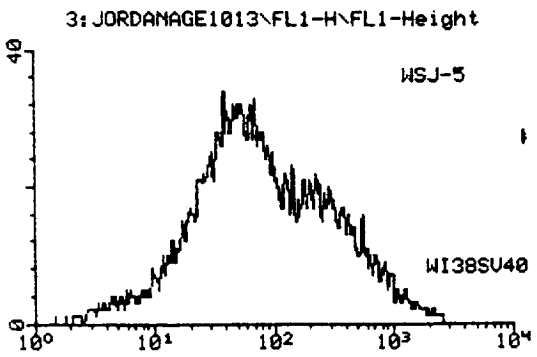
mean = 365.4
NF = 138.2



NUMBER OF CELLS

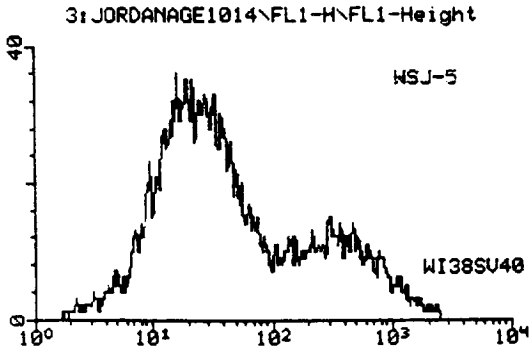
RELATIVE FLUORESCENCE INTENSITY

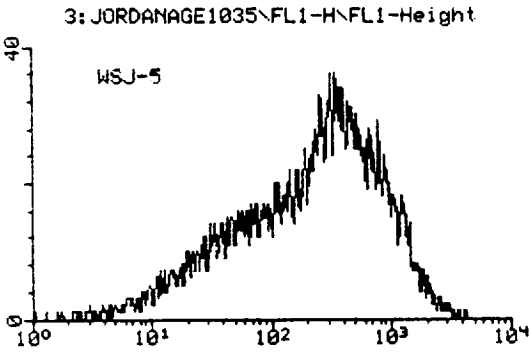
DATE: 10-JAN-95 TIME: 4:38:02



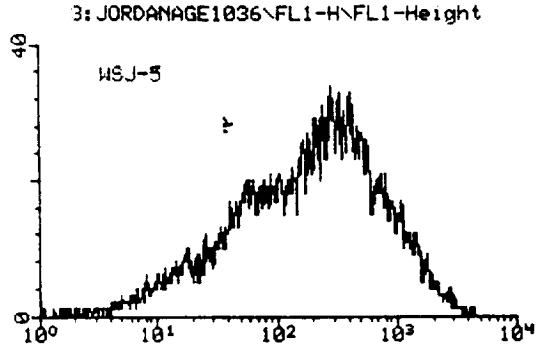
AVGERAGE

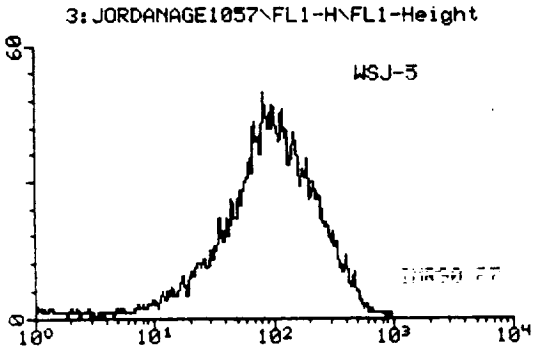
mean = 569.7
NF = 372.4



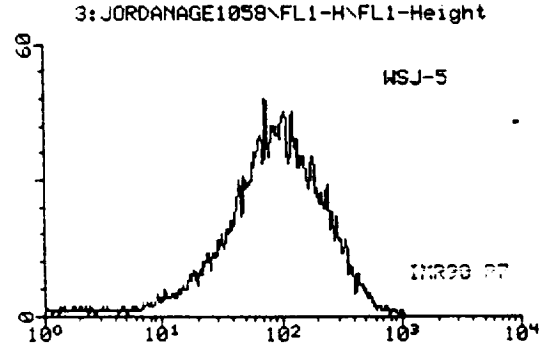


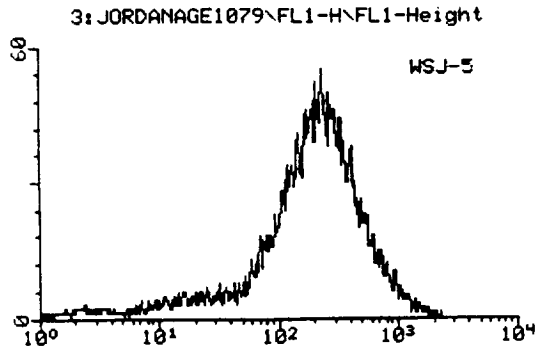
mean = 661.9
NF = 499.5



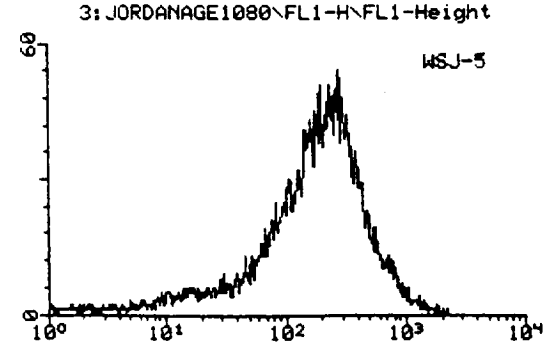


mean = 535.5
NF = 310.0





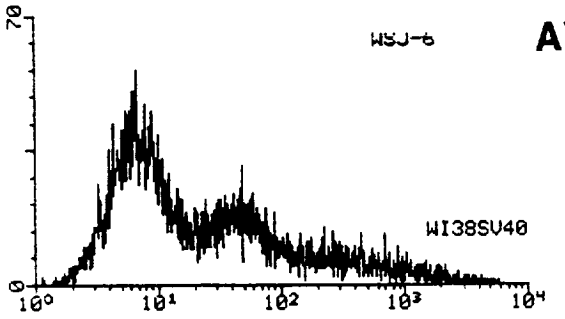
mean = 615.0
NF = 387.8



NUMBER OF CELLS

RELATIVE FLUORESCENCE INTENSITY

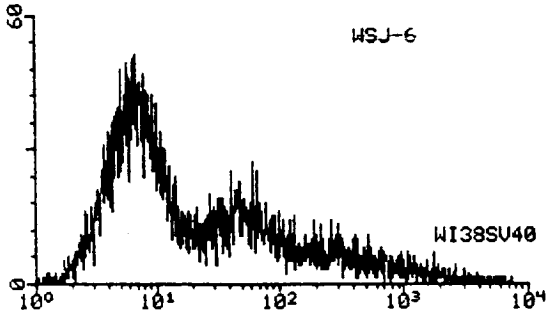
3:JORDANAGE1015\FL1-H\FL1-Height



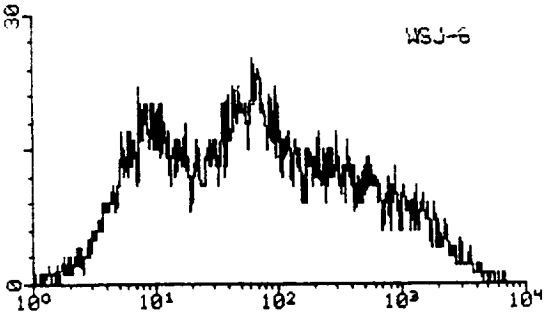
AVGERAGE

mean = 527.6
NF = 330.2

3:JORDANAGE1016\FL1-H\FL1-Height



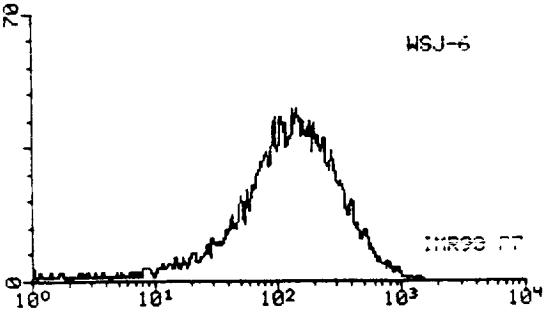
3:JORDANAGE1037\FL1-H\FL1-Height



mean = 634.6
NF = 472.2

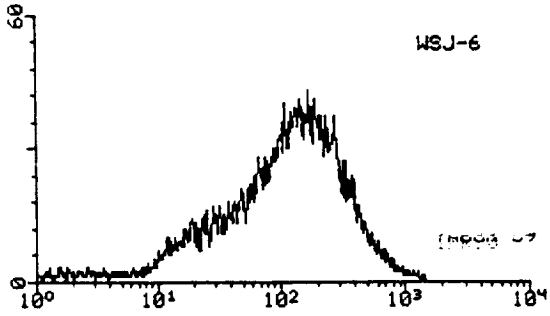
3:JORDANAGE1038\FL1-H\FL1-Height

3:JORDANAGE1059\FL1-H\FL1-Height

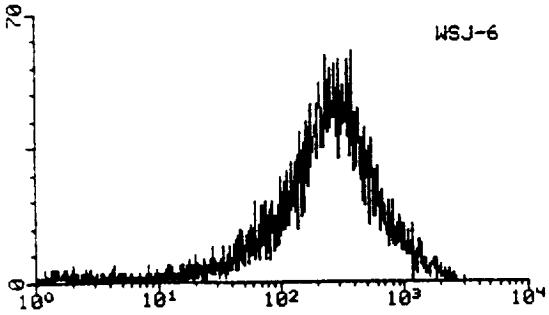


mean = 568.7
NF = 343.2

3:JORDANAGE1060\FL1-H\FL1-Height

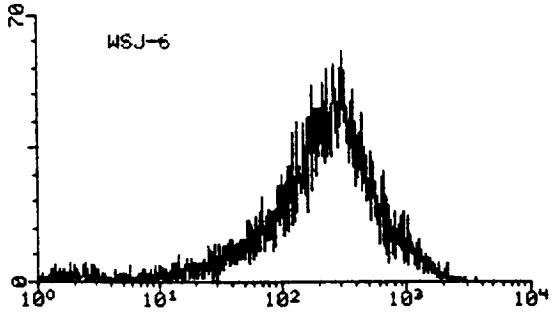


3:JORDANAGE1081\FL1-H\FL1-Height



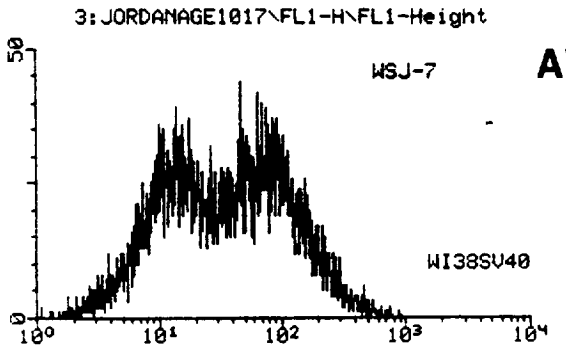
mean = 641.2
NF = 414.0

3:JORDANAGE1082\FL1-H\FL1-Height



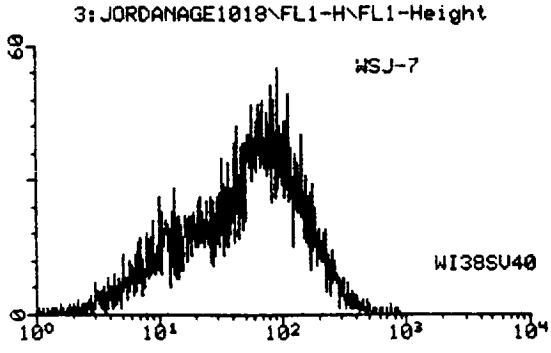
NUMBER OF CELLS

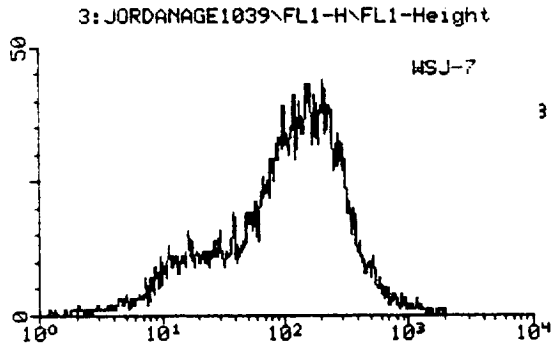
RELATIVE FLUORESCENCE INTENSITY



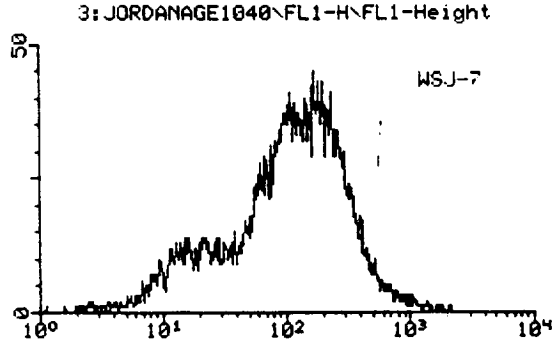
AVGERAGE

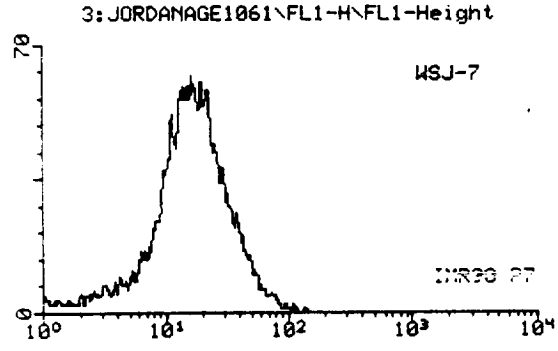
mean = 469.2
NF = 271.9



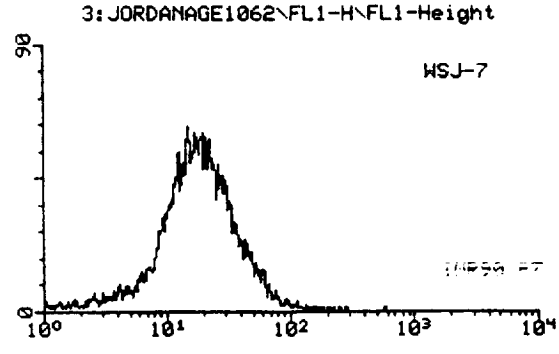


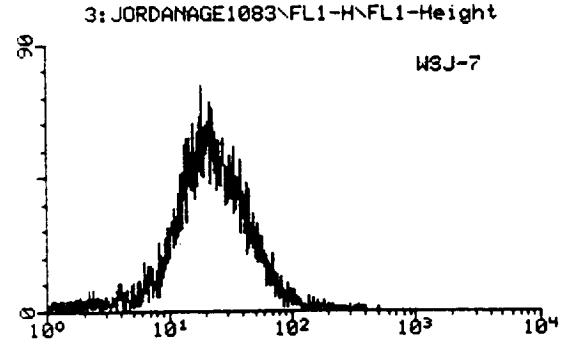
mean = 563.0
NF = 400.6



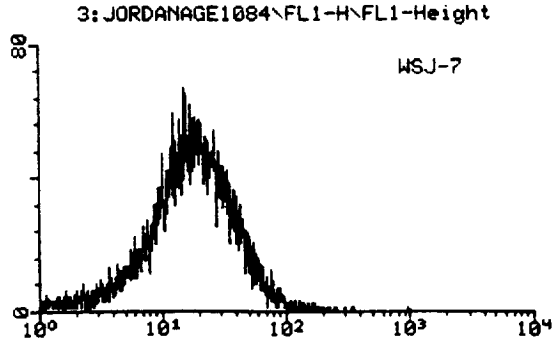


mean = 338.2
NF = 118.0



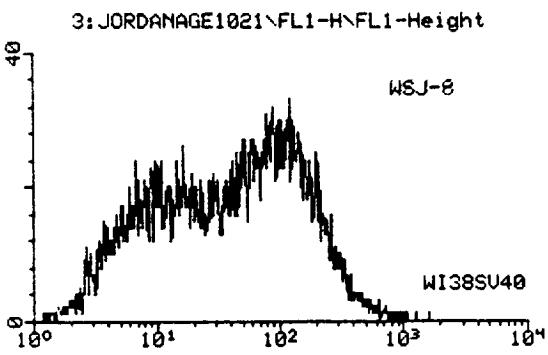


mean = 356.0
NF = 128.8



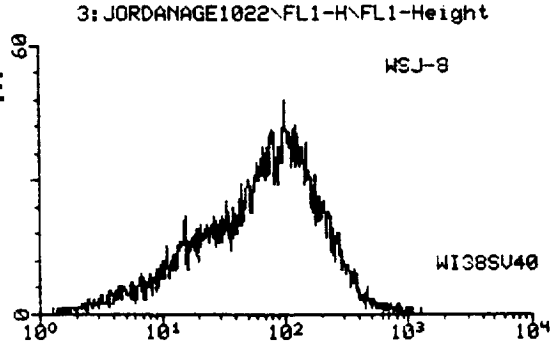
NUMBER OF CELLS

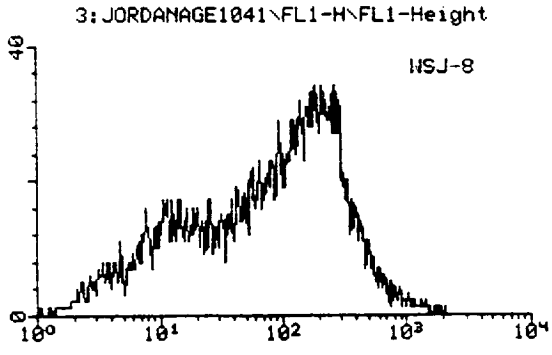
RELATIVE FLUORESCENCE INTENSITY



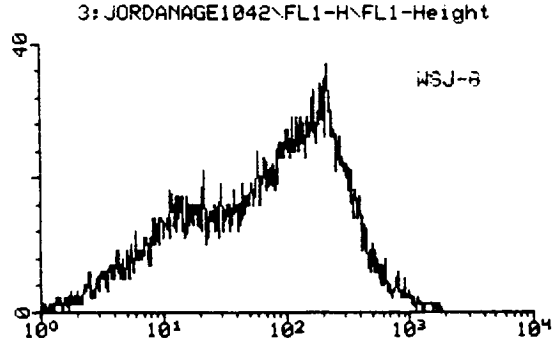
AVGERAGE

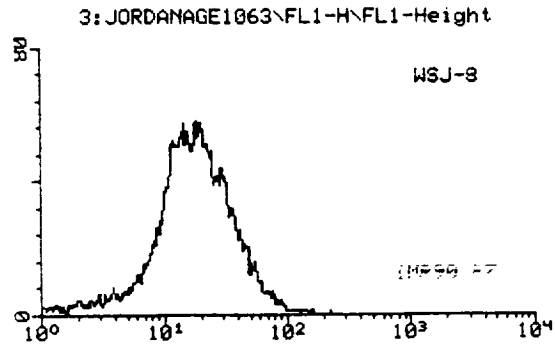
mean = 497.4
NF = 300.0



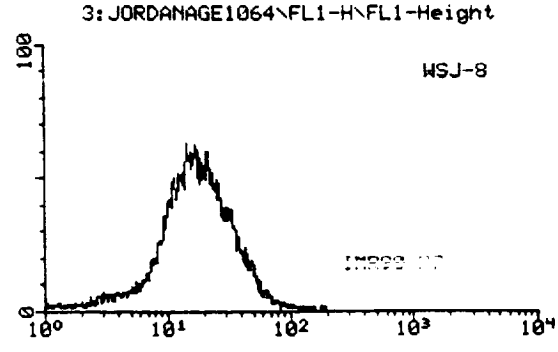


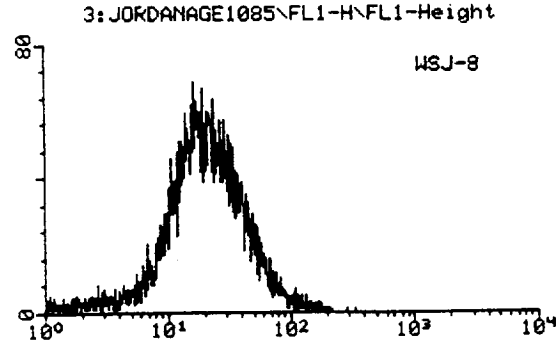
mean = 550.0
NF = 387.6



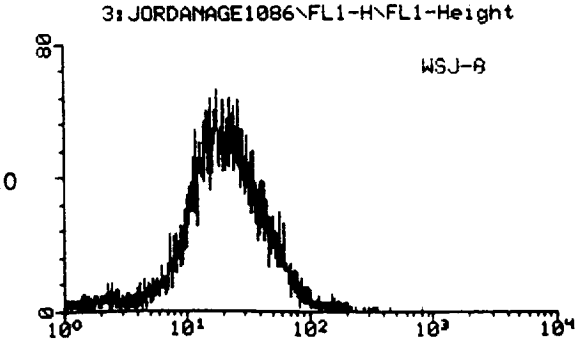


mean = 339.0
NF = 113.6





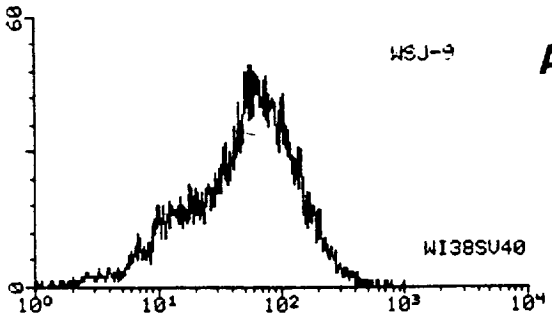
mean = 360.0
NF = 132.8



NUMBER OF CELLS

RELATIVE FLUORESCENCE INTENSITY

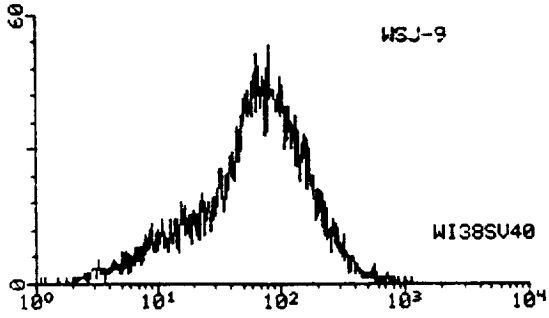
3:JORDANAGE1019\FL1-H\FL1-Height



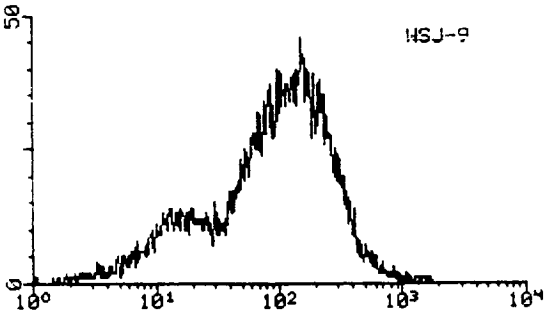
AVGERAGE

mean = 485.5
NF = 288.2

3:JORDANAGE1020\FL1-H\FL1-Height

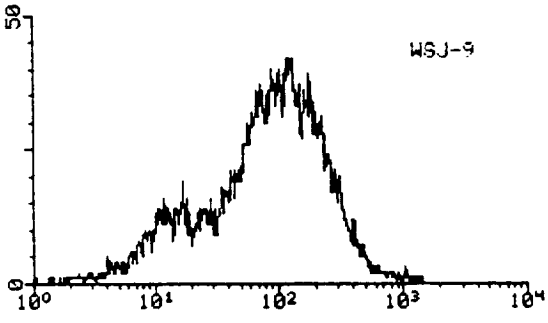


3:JORDANAGE1043\FL1-H\FL1-Height

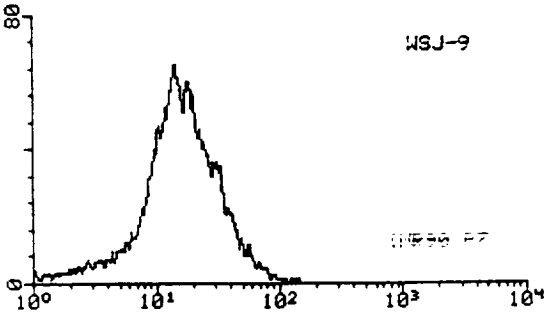


mean = 540.3
NF = 377.9

3:JORDANAGE1044\FL1-H\FL1-Height

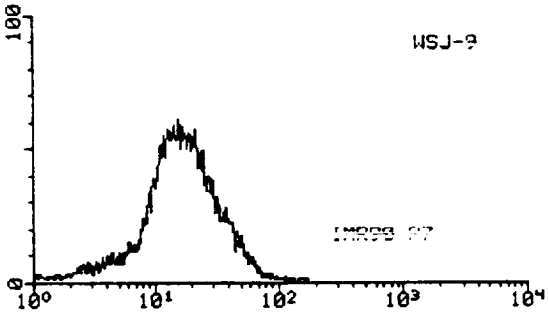


3:JORDANAGE1065\FL1-H\FL1-Height

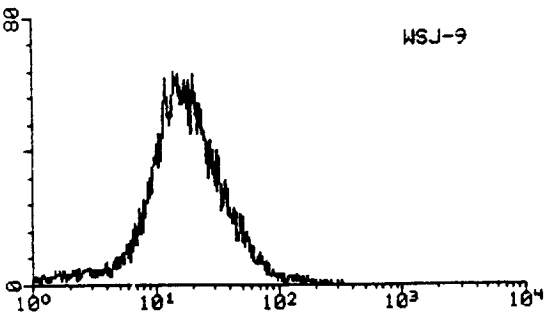


mean = 330.0
NF = 104.5

3:JORDANAGE1066\FL1-H\FL1-Height

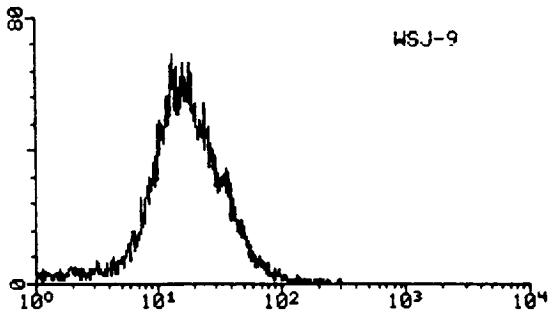


3:JORDANAGE1087\FL1-H\FL1-Height



mean = 340.8
NF = 113.7

3:JORDANAGE1088\FL1-H\FL1-Height



NUMBER OF CELLS

RELATIVE FLUORESCENCE INTENSITY

FLOW CYTOMETRY SUMMARY

(Witt 7B)

ANTIBODY	CELL LINE	MEAN*	NET FLUORESCENCE*
MTS 1.2	WI38sv40	537.2	349.1
	CHO Spinner	254.6	42.6
	CHO PRO-5	365.6	177.5
	2DF*F1	240.5	42.4
WSJ-2	WI38sv40	521.3	333.2
	CHO Spinner	270.0	58.0
	CHO PRO-5	286.9	98.8
	2DF*F1	280.8	82.7
WSJ-3	WI38sv40	533.8	345.7
	CHO Spinner	248.5	36.5
	CHO PRO-5	276.6	88.5
	2DF*F1	265.6	67.5
WSJ-4	WI38sv40	543.2	355.1
	CHO Spinner	275.3	63.3
	CHO Pro-5	296.5	108.4
	2DF*F1	261.4	63.3
WSJ-5	WI38sv40	596.5	408.4
	CHO Spinner	371.6	159.6
	CHO PRO-5	375.9	187.8
	2DF*F1	359.6	161.5

Witt 7B: FLOW CYTOMETRY SUMMARY CONT.

ANTIBODY	CELL LINE	MEAN*	NET FLUORESCENCE*
WSJ-7	WI38sv40	571.3	383.2
	CHO Spinner	249.8	37.8
	CHO Pro-5	329.7	141.6
	2DF*F1	330.8	132.7
WSJ-8	WI38sv40	586.9	398.8
	CHO Spinner	365.6	153.6
	CHO Pro-5	269.6	81.5
	2DF*F1	253.8	55.7
WSJ-9	WI38sv40	635.0	446.9
	CHO Spinner	267.6	55.6
	CHO Pro-5	379.2	191.1
	2DF*F1	371.6	173.5

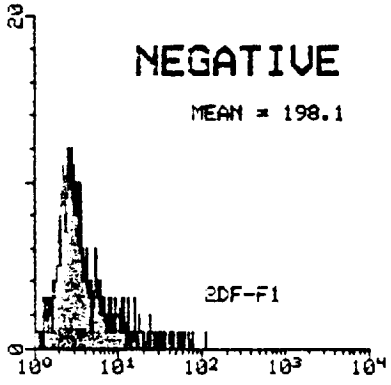
*** All numbers are linear**

Samples were originally run in duplicate, but the tubes were combined when the samples were read on the Facs machine. Also, WSJ-6 was negative on the WI38sv40 cells so these samples were not considered.

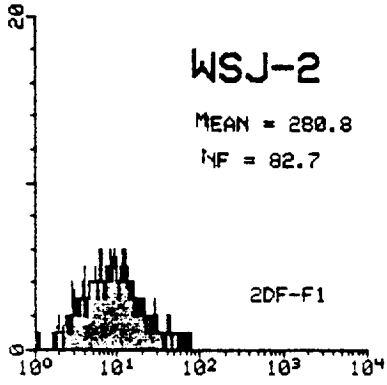
BECTON
DICKINSON

DATE: 2-FEB-95 TIME: 10:19:07

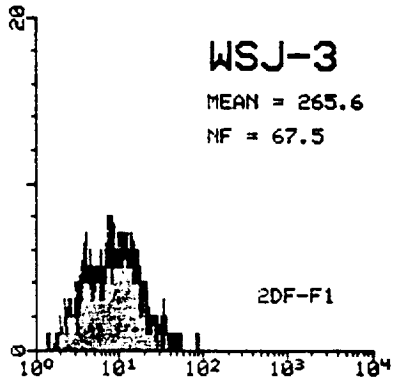
1:WITT7B034\FL1-H\FL1-Height



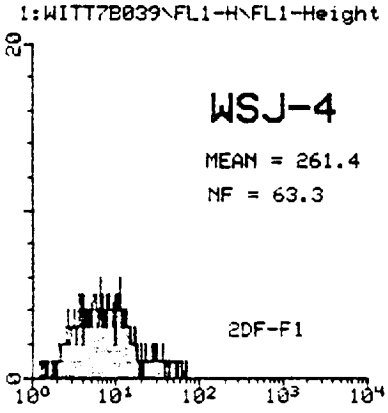
1:WITT7B037\FL1-H\FL1-Height



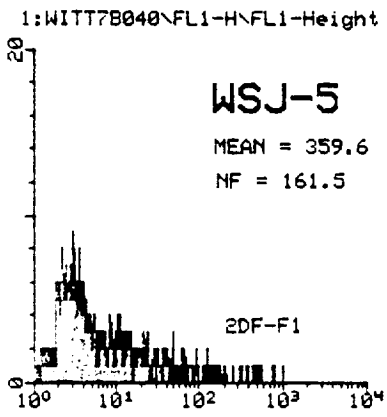
1:WITT7B038\FL1-H\FL1-Height



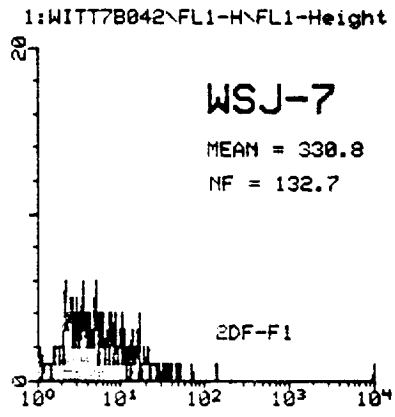
1:WITT7B039\FL1-H\FL1-Height



1:WITT7B040\FL1-H\FL1-Height



1:WITT7B042\FL1-H\FL1-Height



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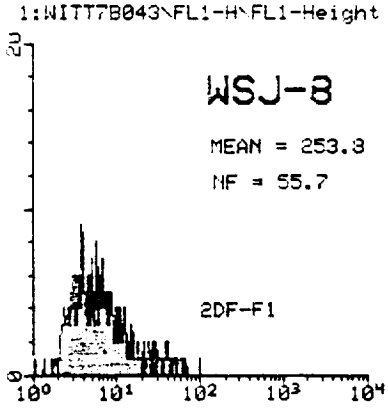

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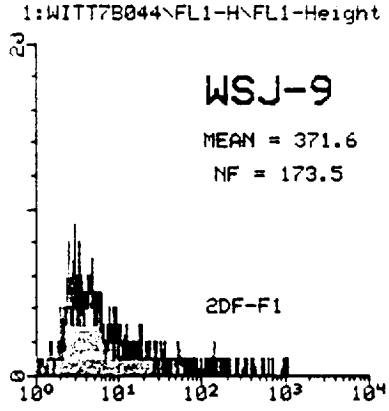

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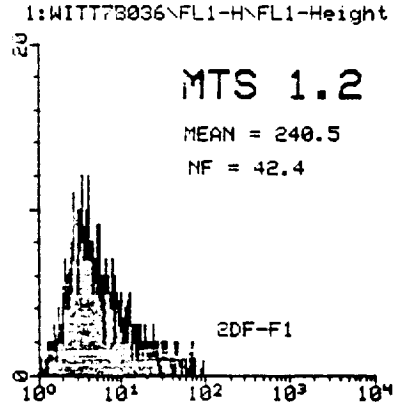

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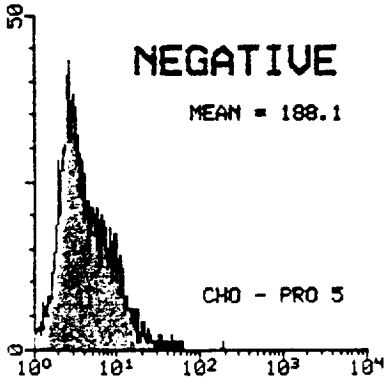

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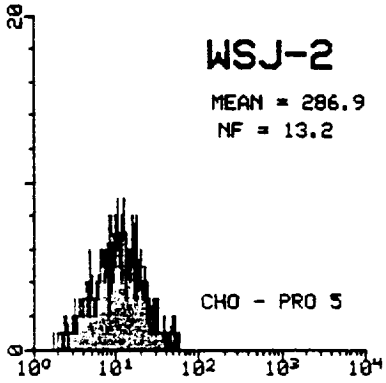

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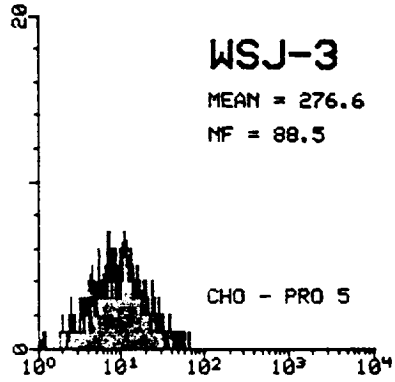

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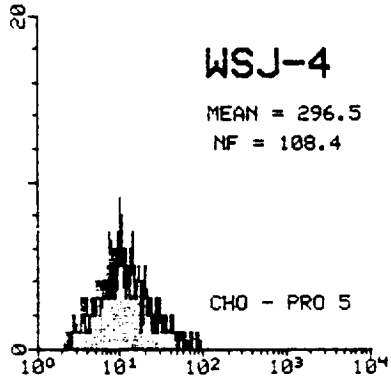

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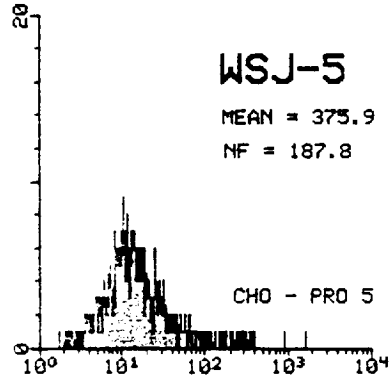

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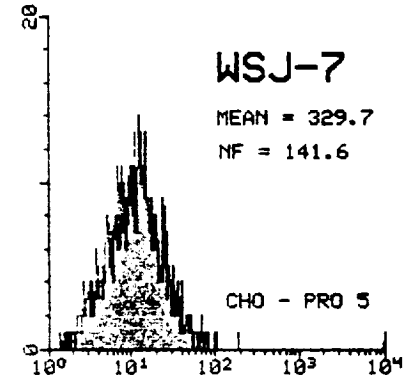

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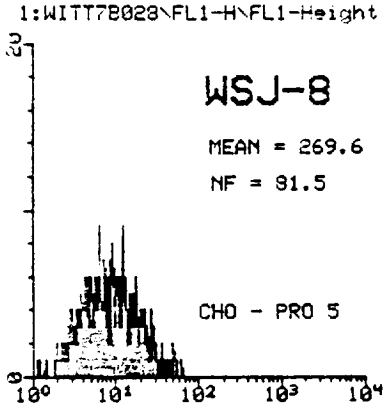

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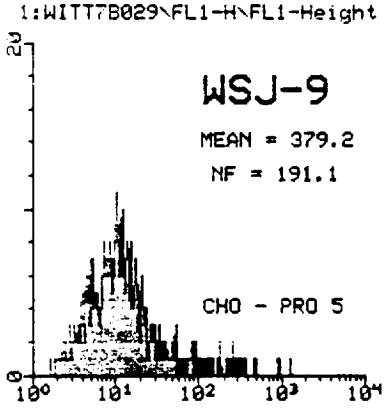

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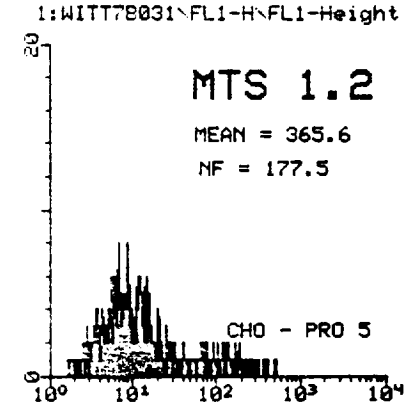

BECTON
DICKINSON

DATE: 2-FEB-95 TIME: 9:42:39

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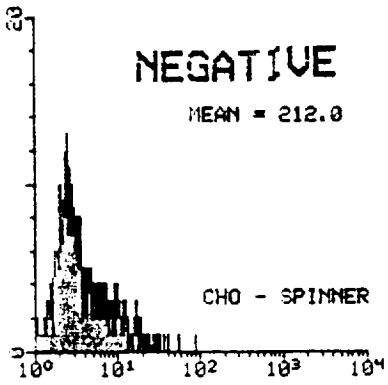

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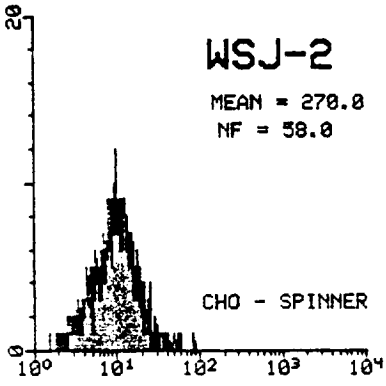

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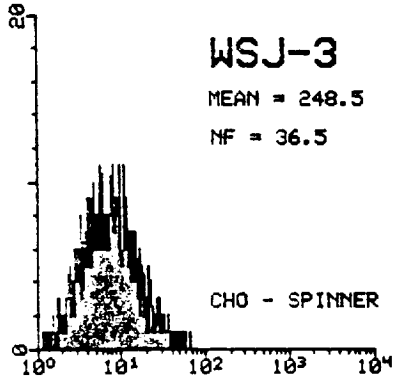

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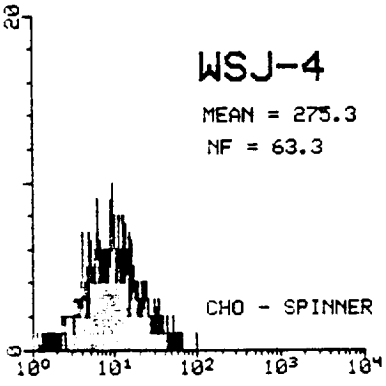

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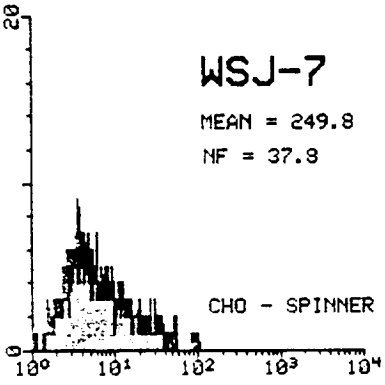

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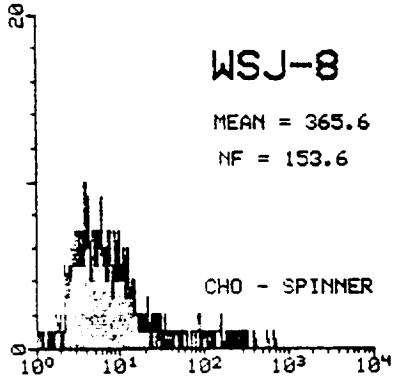

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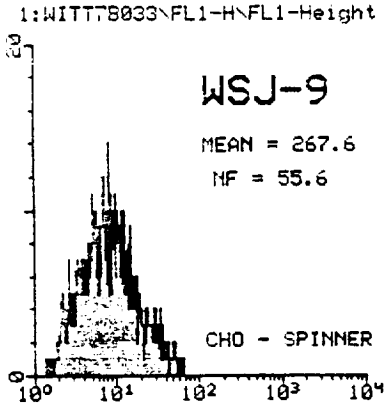

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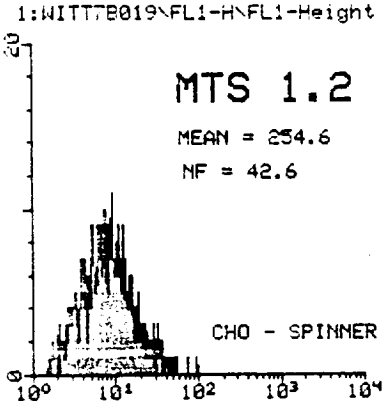

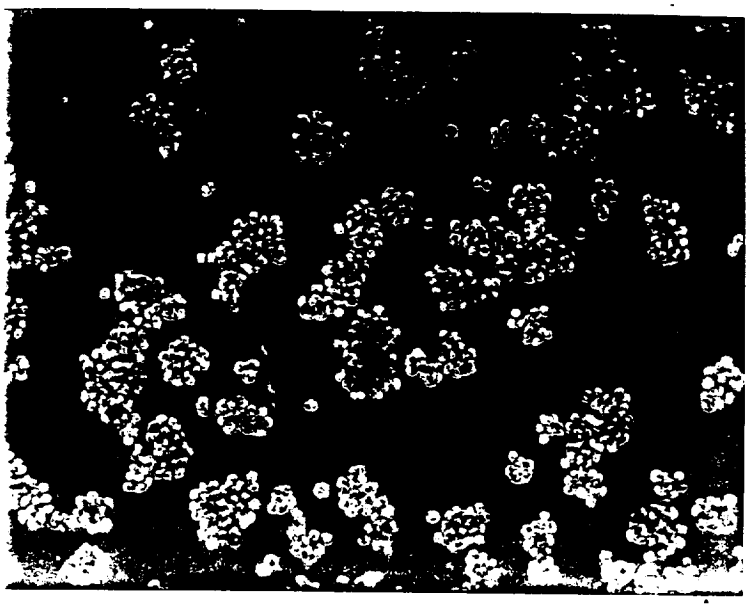

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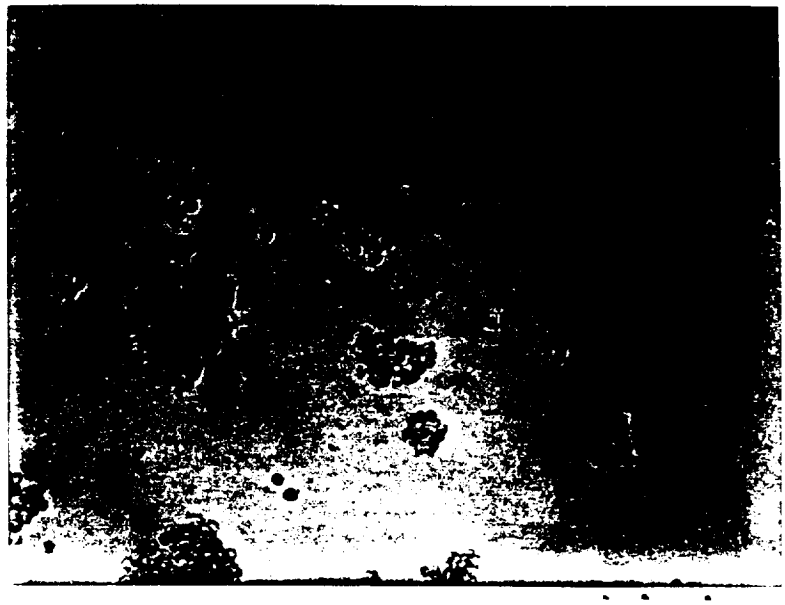
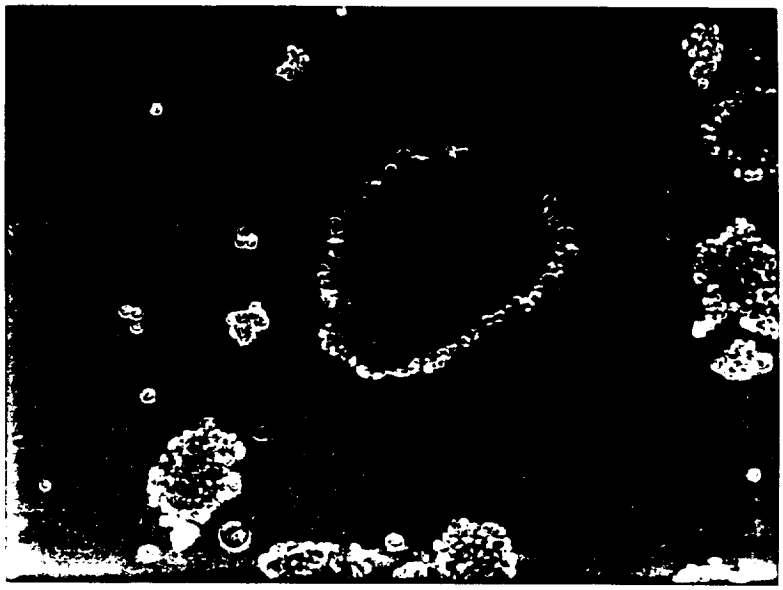


B

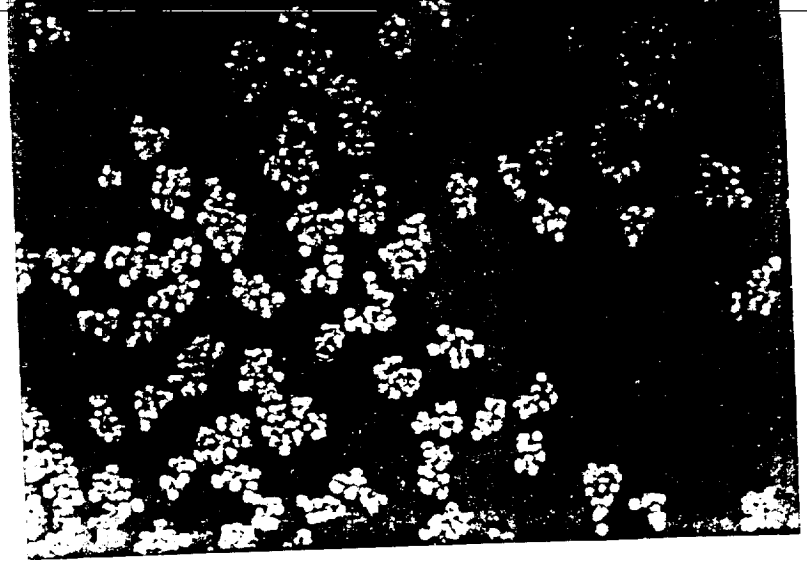
A) Control well contianing 2DF*F1 cells in Iscoves media with 10% Fetal clone at 5 days (10x). B) Well containing 2DF*F1 cells with BSG-1, 3 day supernatant at 5 days (10x).



C

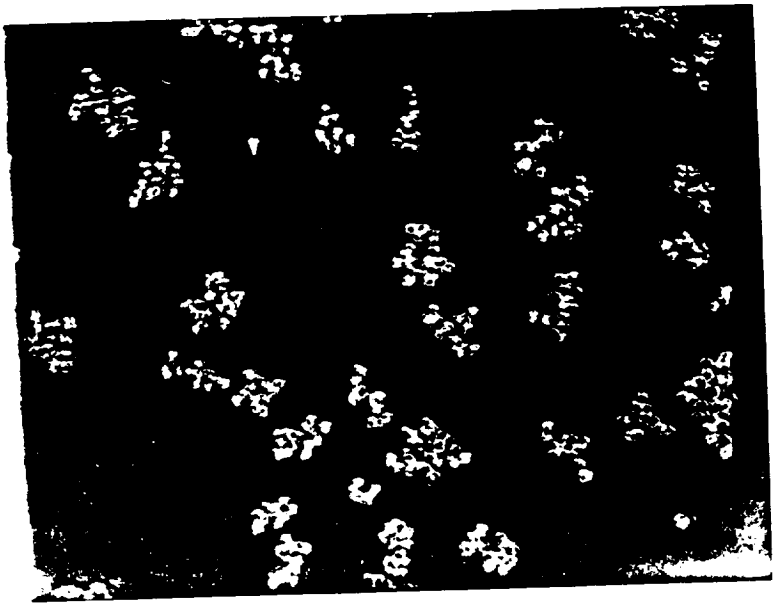
C) Well containing 2DF*F1 cells with BSG-1 concentrated supernatant 1:20 at 5 days (20x).

A

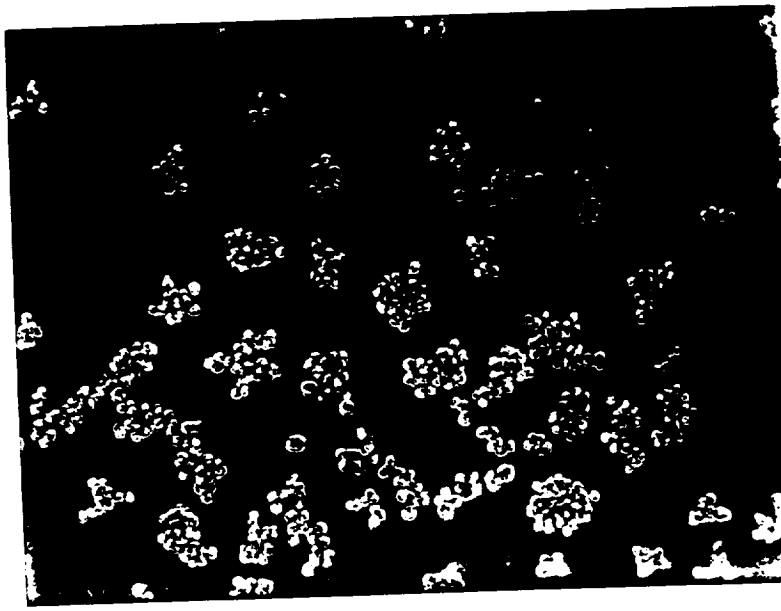


B

A) Control well containing 2DF*F1 cells in Iscoves media with 10% Fetal Clone at 24 hrs. (10x). B) Well containing 2DF*F1 cells with BSG-1, 3 day supernatant at 24 hours (10x).



C

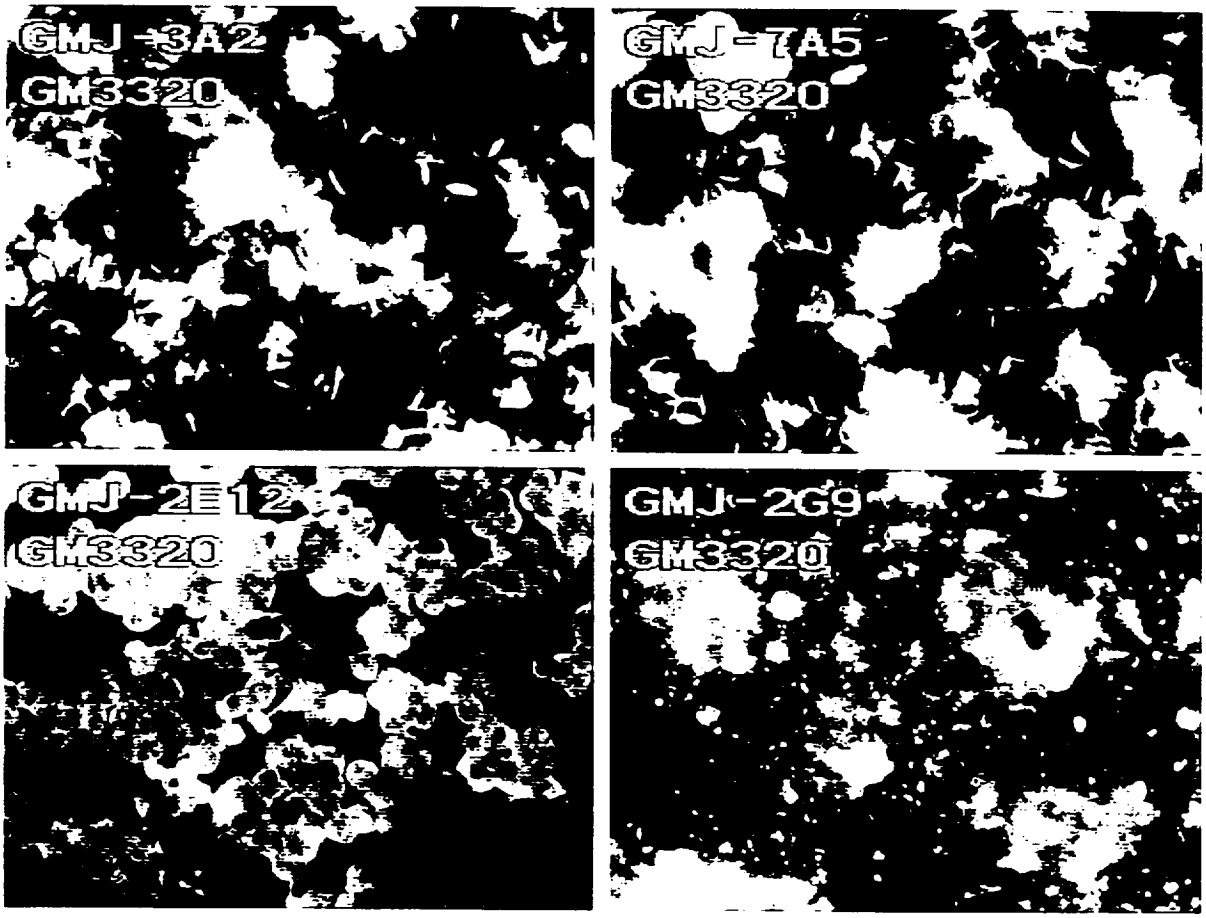


D

C) Well containing 2DF*F1 cells with BSG-1 exhausted supernatant at 24 hours (10x).
D) Well containing 2DF*F1 cells with BSG-1 concentrated supernatant (from 2 liters to 50 mls) diluted in serum-free isoves 1:20 at 24 hrs (10x).


GM3320 FUSION
GMJ-1


GM3320 FUSION
GMJ-2


GMJ-3A2
GM3320
GMJ-7A5
GM3320
GMJ-2E12
GM3320
GMJ-2G9
GM3320